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An Information Storage Mechanism: Calcium and Spines

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This proposal addresses some of the biophysical events possibly underlying fast activity-dependent changes in synaptic efficiency. Dendritic spines in the cortex have attracted increased attention over the last years as a possible locus of cellular plasticity given the large number of studies reporting a close correlation between presynaptic activity (or lack of thereof) and changes in spine shape. This is highlighted by recent reports, showing that the spine cytoplasm contains high levels of actin. Moreover, it has been demonstrated that a high level of intracellular free calcium, Ca^{2+}, is a prerequisite for various forms of synaptic potentiation. We propose a series of plausible steps, linking presynaptic electrical activity at dendritic spines with a short-lasting change in spine geometry. Specifically, we conjecture that the spike-induced excitatory postsynaptic potential triggers an influx of Ca^{2+} into the spine, where it will rapidly bind to intracellular calcium buffers such as calmodulin and calcineurin. However, for prolonged or intense presynaptic electrical activity, these buffers will saturate. The free Ca^{2+} will then activate the actin/myosin network in the spine neck, reversibly shortening the length of the neck and increasing its diameter. This change in the geometry of the spine will lead to an increase in the synaptic efficiency of the synapse. We will discuss the implications of our proposal for the control of cellular plasticity and its relation to generalized attention and arousal.

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Dendritic Spines

Dendritic spines, the principal site of terminations of excitatory afferents in the cerebral cortex, were originally discovered by Ramon y Cajal. Their existence was confirmed by Gray using electron-microscopy (Gray, 1959). Since then, a variety of experimental and theoretical studies, regarding their possible involvement in synaptic plasticity and memory, have appeared. Several groups of investigators have presented evidence that the shape, and not the absolute number of spines, can be modified by experience, presumably in response to variations in the patterns of electrical activity in the presynaptic fibers (Fifkova and van Harreveld, 1977; Lee, Schottler, Oliver and Lynch, 1980). A well studied case is the long-term potentiation (LTP), occuring in granule, CA1 and CA3 cells of the hippocampus, a structure linked to the establishment of memory traces. The afferent fibers to these cells establish contact with the postsynaptic target cell via dendritic spines. The response of the hippocampal cell to a test stimulus is strongly enhanced, following repetitive electrical stimulation to the afferent fibers (Bliss and Lomo, 1973; Alger and Teyler, 1976; Swanson *et al.*, 1981). The basis of this facilitation is a long-lasting increase in synaptic efficiency that begins after a latency of 10-20 sec and may persist for many days or even weeks.

Concomittantly, Fifkova and her colleagues showed that stimulation of the afferent induces a long-lasting enlargement of the spines, especially an increase in spine neck width and a decrease in spine neck length (Fifkova and van Harreveld, 1977; Fifkova and Anderson, 1981; Fifkova *et al.*, 1982).

In a first mathematical analysis of the electrical properties of spines, Rall, on the basis of a simple model, pointed out that variations in the spine neck geometry could effectively change the amplitude of the somatic depolarization evoked by a synapse on the spine (Rall, 1974 and 1978; see also Jack, Noble and Tsien 1975; Perkel 1983). Koch and Poggio (1983a,b), using computer simulations of a reconstructed pyramidal cell and assuming time-varying conductance changes as synaptic input, confirmed and extended Rall's analysis. For a wide physiological range of parameters, small changes in the length and diameter of the spine neck will lead to a large change in the induced somatic potential. For instance, decreasing the spine neck length from 1.6 to $1.0\mu m$, increasing at the same time the diameter so as to leave the total neck surface area constant, enhances the somatic potential induced by the synapse by a factor of two (Koch and Poggio, 1983b).

Crick (1982) conjectured that there might be contractile proteins such as actin/myosin associated with every spine, allowing the spine to change its shape very rapidly. Presynaptic electrical activity, or possibly the conjunction of pre- and postsynaptic activity—as in the Hebb rule—may lead to a rapid contraction of these proteins: the spine twitches. Since actin/myosin in muscle cells contracts very rapidly (tens of ms) a transient spine neck

contraction could occur on a similar time scale, embodying a form of ultra-short memory. Such a quick adjustment of synaptic weights as a function of the previous presynaptic spiking history proves to be very attractive for various models of perception and information storage (van der Malsburg, 1981; Crick, 1984).

Subsequently, different groups of researchers visualized cytoplasmic actin in single neurons, using immunocytochemical methods or labeling with myosin subfragments (Katsumaru *et al.*, 1982; Fifkova and Delay, 1982; Matus *et al.*, 1983; Caceres *et al.*, 1983). While actin seems to be present at all postsynaptic densities throughout the dendritic tree, it concentrates in dendritic spines where it is organized in long filaments oriented parallel to the axis of the spine neck (figure 1). It seems reasonable to speculate that neuronal actin may have a dual role as both structural *and* contractile protein.

Calcium and Calcium Buffers

The role of calcium as a regulator of cell biological properties has long been recognized. From the neurobiological point of view, calcium is currently regarded as one of the key factors in the operation of nerve cells, providing a link between membrane excitation and cellular responses such as secretion, transport or signaling. Since the findings of Katz and Miledi (1967) that the release of transmitter depends on an influx of Ca^{2+} into the presynaptic terminal, it has been suggested that changes in the availability of intracellular free Ca^{2+} might be important for certain forms of short-term synaptic plasticity. Kandel and his group showed that the two forms of plasticity occurring in the sea-snail *Aplysia*, habituation and sensitization, both share a common mode of expression – the modulation of presynaptic Ca^{2+} concentration (Kandel, 1981). The crucial role of calcium for the establishment of LTP in the hippocampus has been underlined by experiments where the level of free intracellular Ca^{2+} in the postsynaptic cell has been severely reduced by injections of EGTA, a calcium chelating agent (Lynch *et al.*, 1983; see also Baimbridge and Miller, 1981). This blocks the development of LTP and strongly suggests that LTP has a postsynaptic locus and that its induction depends on the level of free intracellular calcium. Moreover, elevated extracellular calcium levels induce a long-term increase in synaptic efficacy similar to LTP without any electrical presynaptic stimulation (Turner, Baimbridge and Miller, 1982).

Intriguingly, high concentrations of Ca^{2+} deposits have been found in the spine smooth endoplasmic reticulum and in the sacs of the dendritic spine apparatus (Fifkova, Markham and Delay, 1983). In addition, two of the major neuronal calcium buffers, calmodulin and calcineurin, were likewise identified in high concentration in postsynaptic structures and spines (Wood *et al.*, 1980). Calmodulin (CM), like calcineurin, is a protein with several,

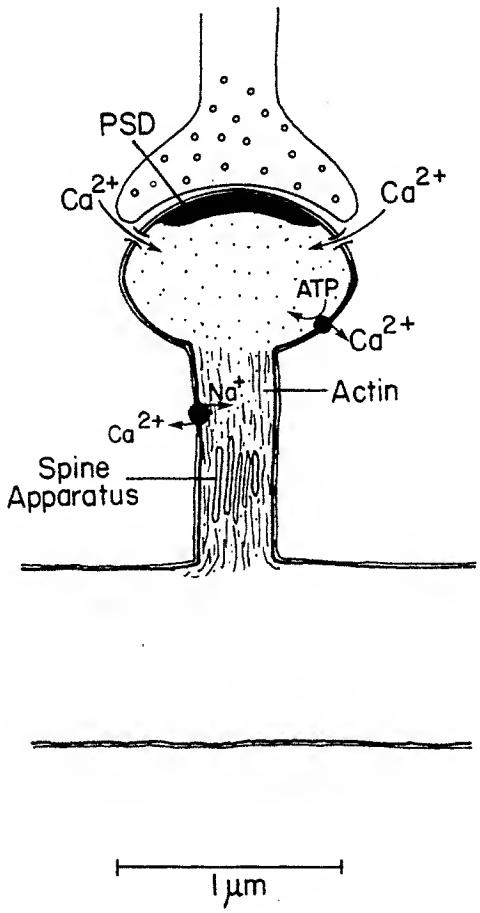

Figure 1. A schematic drawing of a typical dendritic spine in mammalian cortex with some of its constitutive elements. The postsynaptic density (PSD), directly below the presynaptic terminal, is a clump of electron-dense material made up of neurofilaments, actin, fodrin, tubulin, calmodulin, a microtubule-associated protein 2 and other proteins. The spine-apparatus consists of two or three membrane-bound sacs, alternating with thin laminae of dense material. In its typical form this apparatus occurs only in the cerebral cortex. Also shown are the proposed voltage- or neurotransmitter-dependent calcium channels, the sodium-calcium exchange pump (in its normal mode of operation) and the ATP driven calcium pump. The scale is only approximate.

probably 4, Ca^{2+}-specific binding sites. Once CM becomes fully activated, for which all four calcium sites must be occupied ($Ca_4 \cdot CM$), it becomes a powerful activator of various enzyme systems and proteins, inducing for instance actin/myosin contractions (Klee and Haiech, 1980; Cheung, 1982).

The link between calcium-activated processes and membrane excitability is provided by voltage-dependent calcium channels. The presence of such channels has been amply documented in a variety of preparations, for instance in the nodal regions of the heart (Brown, Giles and Noble, 1977), in hippocampal neurons (Schwartzkroin and Slawsky, 1977)

and in Purkinje cells *in vitro* (Llinas and Hess, 1976; Llinas and Sugimori, 1980), olivary (Llinas and Yarom, 1981) and thalamic neurons (Jahnsen and Llinas, 1984). In all of these neuronal systems, the main evidence for a Ca-dependent conductance are high-threshold Ca-spikes, believed to originate in the dendritic tree. These channels are activated by membrane depolarization, have varying activation thresholds, and show little or very slow voltage-dependent inactivation (Tsien, 1983). Some channels do, however, show increasing inactivation as a function of $[Ca^{2+}]_i$. Calcium channels, especially those in the heart, are known to respond to a wide variety of neurohormones. Of potential great interest to us is the excitatory action of β-adrenergic agents on calcium channels.

To summarize, dendritic spines, the major site of contact between axons and neurons in the cortex, seem to be able to change their shape as a function of presynaptic activity, contain proteins capable of mediating contraction and show a very large Ca^{2+} regulating capability. Thus, the groundwork seems to be laid for a detailed mechanism, linking the biophysical events at a spine upon arrival of a presynaptic spike to synaptic plasticity and ultimately to memory.

Recently, in two elegant accounts, Eccles (1983) and Lynch and Baudry (1984) develop and present experimental evidence for two differing hypotheses concerning the biochemistry and biophysics possibly underlying long-term changes in synaptic efficiency. While both involve as a first step the influx of Ca^{2+} into dendritic spines, they differ substantially as to the following steps. In particular, both proposals disregard the possible contribution of a modifiable spine geometry towards establishing short-term memory.

The Basic Hypothesis

We will proceed to sketch an hypothetical picture of the events occuring at a dendritic spine following sustained presynaptic activity.

- Presynaptic electrical activity results in an influx of Ca^{2+} ions via voltage-dependent channels into the spine head.
- This intracellular free calcium will be rapidly bound to the high affinity calcium buffers such as calmodulin and calcineurin.
- The high buffering capability of the spine cytoplasm, together with membrane pumps and the diffusion of Ca^{2+} into the dendrite, will work to keep $[Ca^{2+}]_i$ and $[Ca_4 \cdot CM]$ low in response to moderate presynaptic activity. If however, the presynaptic activity exceeds a critical amount, the calcium buffers will saturate.
- Subsequently, and for continuing presynaptic input, the concentration of free Ca^{2+} — and therefore of $Ca_4 \cdot CM$ — will rise very rapidly, leading to a contraction of the

actin/myosin present in the spine neck. This contraction, lasting as long as the high levels of $[Ca_4 \cdot CM]$ persist, is functionally equivalent to a fast modification of synaptic weight.

- Moreover, activated calmodulin associated with the spine membrane cytoskeleton sets off two different chain of events. Binding of $Ca_4 \cdot CM$ to the microtubule associated proteins causes disassembly of these microtubules. Activated calmodulin also interacts with the fodrin-actin cytoskeleton network by binding to the fodrin. Both mechanisms lead to a loosening of the spine cytoskeleton, i.e. the spine becomes flabby. This, in conjunction with the actin/myosin contraction, may allow for more permanent spine shape changes to occur.

Each of the above steps has been shown to occur in different neuronal and nonneuronal systems. Moreover, the ultrastructural evidence about spines seems consistent with this sequence of events. They could thus be one biophysical mechanism underlying short-term information storage in the central nervous system. In the next section we will discuss in more detail each of the individual processes.

The Biophysics of Spines

Activity-dependent Calcium Influx

Our single most important requirement is that presynaptic activity leads to an elevated level of free intracellular calcium. The mechanism most likely to underlie such an increase is a voltage-dependent calcium entry through calcium channels. Due to the expected high input impedance of spines, even small synaptic conductance changes will cause a very large potential change in the spine head, easily reaching 30 or 40 mV (Koch and Poggio 1983b). Thus, even high threshold calcium channels will be activated, resulting in a rapid influx of Ca^{2+} into the spine—as first proposed by Perkel and Brown, 1982—down an electrochemical gradient that is as high as -200 mV, the inside Ca^{2+} concentration being $10^{-7}M$ (extracellular Ca^{2+} concentration is usually around $10^{-3}M$). An alternative to this scheme are neurotransmitter-activated channels which would allow either unselective passage of both Na^+ and Ca^{2+} ions or only Ca^{2+} ions.

Two other possible mechanisms for raising $[Ca^{2+}]_i$ are the sodium-calcium exchange pump and voltage- and calcium-dependent release from intracellular calcium storage sites. Repetitive presynaptic stimulation will lead to high intracellular Na^+ levels, driving the sodium-calcium exchange in reverse. In this mode Ca^{2+} will be able to enter the cell, driving out Na^+, due to the reduced Na^+ gradient (DiPolo and Beauge, 1983). In muscle an intracellular organelle system, the sarcoplasmic reticulum, stores and releases Ca^{2+} in

a voltage- and/or calcium- dependent fashion. In non-muscle cells, it is believed that the endoplasmic reticulum is the main regulator of cell calcium (Somlyo, 1984; see also Neering and McBurney, 1984). Because of the similar membrane structure and calcium accumulating ability of the spine apparatus, Fifkova *et al.* (1983) suggested a stimulus induced release of Ca^{2+} from this structure. All of these mechanisms may of course contribute simultaneously to the postulated stimulus-dependent rise in free intracellular calcium.

Calcium Buffers

The calcium buffers fall into three distinct populations, differing in their affinities for calcium and in their concentrations. High-affinity buffers will bind calcium significantly at calcium concentrations as low as $0.5\mu M$. One of the best characterized of these is calcineurin, concentrated in postsynaptic densities (Wood *et al.*, 1980; Klee and Haiech, 1980). Calmodulin is the major medium- to high-affinity buffer, having a dissociation constant $K_d \approx 10^{-5}M$ (Klee and Haiech, 1980). It is associated in high amounts with postsynaptic densities and cytoplasm (Grab, Carlin and Siekevitz, 1980; Wood *et al.*, 1980). The last group of buffers (low-affinity $K_d > 5 \cdot 10^{-4}M$), is, among others, comprised of ATP, amino acids, membrane phospholipids and inorganic buffers. They are found throughout neuronal cytoplasm in comparatively high concentrations, only contributing to buffering at the upper limit of physiological calcium concentrations.

Upon entry of calcium from the extracellular space, it will bind within a fraction of a millisecond to the high- and medium-buffers. Due to the rapid calcium binding kinetics most of the entering calcium is buffered at sites very close to the membrane cytoskeleton before diffusing into the cytoplasm (Zucker and Stockbridge, 1983). Only when these sites start to saturate do substantial amounts of Ca^{2+} diffuse into the spine neck. Several other factors will contribute towards keeping the $[Ca^{2+}]$ in the spine neck low. The sodium-calcium exchange pump (now working in its normal mode), the ATP driven calcium pump, the likely sequestration of Ca^{2+} by the spine apparatus and the diffusion of Ca^{2+} into the dendrite will all delay the point at which $[Ca^{2+}]_i$ becomes high enough to cause occupation of all four calmodulin Ca^{2+} binding-sites (Klee and Haiech, 1980).

Fast, Transient Changes in Spine Geometry

Once Ca^{2+} influx succedes in saturating the buffers, $[Ca_4 \cdot CM]$ can rise quickly with further influx of Ca^{2+}. The fully activated calmodulin will now cause shortening of the actin/myosin filaments. Fast contractions of actin in nonmuscle cells are usually mediated by the interaction of actin and myosin filaments. Although nothing is known about the distribution of myosin in postsynaptic structures, its presence in the spine neck seems

likely, given the oriented and crosslinked nature of the actin array (Fifkova *et al.* 1983) which is present in similar concentration to that in adjacent smooth muscle cells (Matus *et al.* 1983). Due to the strategic location of the actin in the spine neck, shortening these filaments increases the neck diameter and decreases at the same time the neck length. As the analysis by Rall (1974, 1978) and by Koch and Poggio (1983a,b) shows, this can reduce the spine head input impedance, leading to more current flowing into the spine - and therefore to the soma - during synaptic input: the synapse has enhanced its somatic weight. The time course of contraction - and of synaptic enhancement - is governed by the rate at which calcium becomes unbound from the calmodulin, which in turns depends on the concentration of free intracellular calcium. A hypothetical time course of $[Ca^{2+}]$ and $[Ca_4 \cdot CM]$ for prolonged synaptic input is shown in figure 2. The actin/myosin contraction may last, depending on the rate-constants assumed and on the level of $[Ca^{2+}]_i$, up to an appreciable fraction of a second. As soon as the contraction ceases, the spine assumes its original shape due to the rigidity and elasticity of the cytoskeleton. A brief transient increase in $[Ca_4 \cdot CM]$ may possibly be induced by a short-lasting high-frequency burst of presynaptic spikes. The relatively small rise in $[Ca^{2+}]$ and $[Ca_4 \cdot CM]$ would mean that the corresponding synaptic potentiation would last only very briefly, possibly in the hundred millisecond range (Crick, 1982). To conclude, this mechanism may give rise to a short lasting, activity dependent, postsynaptic enhancement of synaptic transmission.
Note that the calcium buffers essentially act to buffer the onset of the synaptic enhancement and protect the system from noise induced by single spikes.

Persistent Changes in Spine Geometry

But how can this transient enhancement be made more permanent? A major component of the cytoskeleton is tubulin, the protein that polymerizes into structural microtubules. So called τ proteins, one species of microtubule associated proteins, are essential for the tubulin to assemble. Activated calmodulin binds in the presence of Ca^{2+} to the τ proteins, preventing their association with microtubules and promoting their disassembly. In view of the central role of microtubules in the maintainance of the cytoskeleton and in cell shape determination, we propose that in spines activated calmodulin will loosen the cytoskeleton by this mechanism (Kakiuchi and Sobue, 1981). When free calcium levels subside after a period of intense presynaptic activity, the τ proteins become available again for the assembly of microtubules stabilizing the new spine shape. Calmodulin will also interact strongly with another cytoskeleton protein, fodrin (Kakiuchi and Sobue, 1983). Fodrin, or brain spectrin, a major protein responsible for giving the neuronal membrane its rigidity, normally provides a strong network in the cytoskeleton by crosslinking with actin filaments. It has been proposed that spine fodrin is degraded by a calcium-activated protease, calpain I (Lynch and Baudry,

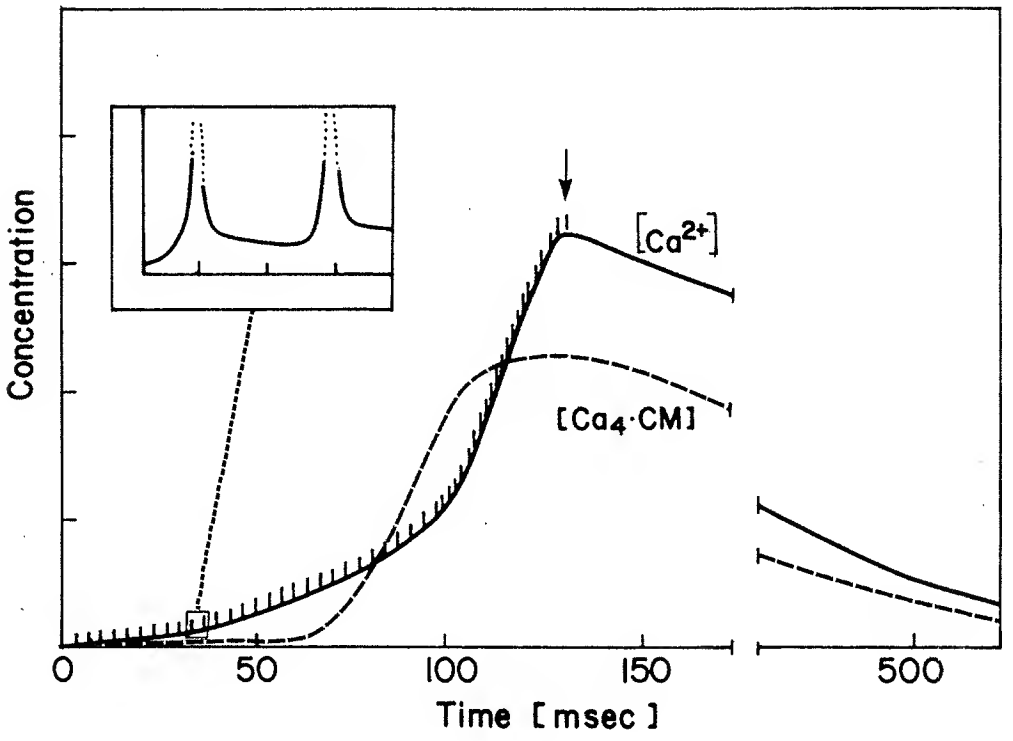

Figure 2. A hypothetical time-course of free intracellular calcium and of activated calmodulin in a dendritic spine during prolonged presynaptic activity (in this case at 300 Hz for 120 ms). The stimulus-induced influx of Ca^{2+} is buffered within a fraction of a ms. Each spike will, however, lead to a small increase in $[Ca^{2+}]_i$ and therefore to increased buffering of Ca^{2+} to the calmodulin complex (see inset). Once the presynaptic input ceases, both $[Ca^{2+}]_i$ and $[Ca_4 \cdot CM]$ decay slowly, their time-course being essentially limited by the low calcineurin off-rate constant (Klee and Haiech, 1980). A sudden increase in intracellular calcium during and following repetitive presynaptic stimulation in hippocampal neurons has been reported (Morris, Krnjevic and Ropert, 1983). Detailed computer simulations, describing the time-course of calcium and the various calcium buffers in a 3-compartment model as a function of presynaptic activity, confirm such a behavior (Robinson and Koch, 1984).

1984; Siman, Baudry and Lynch, 1984). We wish to point out another possibility. The strong binding of activated calmodulin *directly* to fodrin might cause a change in the fodrin-actin network such that additional loosening of the cytoskeleton occurs. This would be easily reversible in low calcium. Disruption of either the microtubule components or the spectrin network in the cytoskeleton of erythrocytes has been shown to be sufficient to cause cell shape changes.

All of the proposed mechanisms, i.e. actin/myosin contraction, disassembly of microtubules and loosening of the fodrin-actin network, are mediated by activated calmodulin. The critical role of calmodulin for establishing synaptic plasticity has been demonstrated by experiments

where post-synaptically injected TFP, an inhibitor of activated calmodulin, abolished LTP (Finn, Browing and Lynch, 1980).

Hebb Synapses

In our proposed mechanism the modification of synaptic transmission at cortical synapses occurs at a postsynaptic site. This modification does therefore not only depend on presynaptic electrical activity, as for sensitization in *Aplysia*, but also on the electrical activity in the postsynaptic cell, conforming to Hebb's rule. The most likely locus for such an interaction between pre- and post-synaptic activity is the stimulus-induced calcium rise. If the calcium influx is mediated by voltage-dependent channels, reducing the voltage in the spine would then prevent the channels from opening. Interestingly, between 5 and 20% of the total number of cortical spines show a pairing of both symmetrical and asymmetrical, i.e. probably excitatory and inhibitory, synapses (Scheibel and Scheibel, 1968; Sloper and Powell, 1979; Somogyi *et al.*, 1983). As Koch and Poggio (1983b) pointed out, such a local circuit would be ideal for inhibiting the excitation without causing inhibition of the electrical activity in the rest of the cell (this is especially true if the inhibition has a reversal potential close to the resting potential of the neuron). Activating the inhibition would not only serve to reduce the evoked dendritic depolarization but would also prevent any synaptic facilitation taking place. The cell only "learns" if presynaptic spiking activity does not coincides with postsynaptic inhibitory activity. The strength of the veto operation would depend on the threshold of the voltage-dependent calcium current. The alternative to this very specific veto operation is a generalized hyperpolarization of the dendrite, making the "learning rule" less specific. If simultaneously present inhibition can prevent the enhancement of synaptic efficiency, simultaneous excitatory input from different, but neighboring, afferents is expected to increase the strength and persistence of the potentiation, an experimental finding known as associative LTP (Lee, 1983; Barrionuevo and Brown, 1983).

Cortical Plasticity and Attention

One can ask at this point whether our proposed mechanism could also be responsible for the experience dependent modification of neuronal connectivity observed in mammalian cortex during early postnatal life. Receptive field properties of neurons in the visual cortex, for example orientation selectivity or binocularity (Wiesel and Hubel, 1963), are especially susceptible to the type and quantity of visual experience gained during the "critical" or "sensitive" period. One suggestion as to the neural factor causing cortical plasticity entails the correlation of the visual input with some gating signal, originating from the midbrain

reticular formation and/or the medial thalamic region (Singer 1982; see also Sillito, 1983). Singer's thesis is that synaptic modification occurs only when the dendritic depolarization of the postsynaptic cell exceeds a critical level, requiring the conjunction of a "gating input" with the visual input. Neglecting for the moment any possible voltage-dependent inactivation of calcium channels, such a depolarization would increase the calcium influx into the spine during presynaptic activity, causing an earlier onset of saturation of the calcium buffers. The neuron would react sooner to changes in its synaptic input than without the gating input.

A second, more intriguing, possibility is suggested by the data of Kasamatsu and Pettigrew (1979). They provided evidence that the diffuse noradrenergic projection originating from the locus coeruleus is a critical ingredient for cortical plasticity. Heart physiologist's have shown on the other hand that a positive inotropic effect of β-adrenergic agents on the heart is mediated by an increased influx of calcium during the plateau phase of the cardiac action potential (Reuter, 1974). The biophysical basis of this enhanced influx is most likely an increase in the mean open time and a decrease in the interval between bursts of single calcium channels (Reuter *et al.* 1982). Plasticity in the cortex, known to posses β-adrenergic receptors, could be facilitated by such a mechanism. During development, locus coeruleus axons might release noradrenaline which will diffuse into the extracellular space surrounding the target neuron. In the absence of presynaptic input noradrenaline will have little effect on the postsynaptic cell. A presynaptic spike will, however, cause a greater Ca^{2+} influx into the spine than before, leading to a faster synaptic modification. Since the midbrain has been implicated in arousal and attention, one could suppose that this scheme could be operating throughout the whole lifetime of an individual, providing under certain circumstances faster information storage capabilities than usually.

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